Museum Preparations. The Preservation of Green Colours in Botanical Specimens Exposed to Light

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VIII.—MUSEUM PREPARATIONS.

The Preservation of Green Colours in Botanical Specimens Exposed to Light.

J. W. H. Trail.

The alteration caused in green parts of plants after exposure to light for some time, whether these are dried or in preservative fluids, renders them unsightly, and deprives them of much of their value by obscuring or obliterating the distinction in colour between the assimilating tissues and the other parts of the specimen. With specimens in fluid, especially when preserved in alcohol, it is customary to bleach them until all parts are deprived of colour before they are admitted to the shelves of museums. Such bleached specimens are indeed preferable to the brown ones that are apt to result where alcohol is used without previous bleaching; and they have the further advantage, when bleached, of not discolouring the preservative fluid. But the uniformity of tint, whether brown or colourless, greatly lessens the usefulness of even the most carefully prepared specimens, especially in an educational museum, and I have sought by various methods to preserve the green colours at least sufficiently to indicate the important difference in function between the green parts and the other organs of plants. The various methods in use and the various solutions recommended as preservatives were tried, and, while partially successful in certain cases, all were unsatisfactory in results, or difficult to employ and liable to fail.

I had tried acetic acid as a preservative fluid; and found that although specimens, especially small bodies, such as galls, hermetically inclosed in glass tubes, retained their form in it they became discoloured. The effect of the salts of copper on the colour of vegetables preserved for food was known to me; but the value of copper as an aid in the preparation of permanent specimens for teaching, and for exhibition in museums, suggested itself to me more clearly after reading Dr. E. Schunck's papers on the chemistry of chlorophyll. I sought to obtain the formation within the green parts of the specimens of the compounds of

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chlorophyll with copper that Dr. Schunck had shown to remain green when exposed to light. I at first made use of acetate of copper dissolved to saturation in strong acetic acid, one part of the solution being diluted with four of water. In this solution the specimens were allowed to steep for a month or more, they were then washed in clean water for a few minutes until clear of all trace of surface deposits of acetate of copper; and they were then transferred to any preservative fluid desired. A short account of the process is given in a report on methods employed by me for preservation of specimens submitted to a committee of the British Association, and printed in the Report of the meeting in Liverpool in 1896. As stated in that account, the method was found to give excellent results in certain cases, the green being quite permanent, and almost natural, except for a bluish tinge in it.

But in other cases the specimens became brown while being steeped in the solution of acetate of copper; and as the browning seemed worst in those plants such as Oak, that contained much tannin, I supposed for a time that it was a compound of tannin formed with the copper that caused the discolouration. I continued to use the method, trying it with different groups of plants, and varying the details as to concentration. The more rapid and effective absorption of certain dyes and other substances by dead than by living protoplasm suggested the advantage of killing the protoplasm in the specimens as rapidly as possible. It was found that in the preservation of *Spirogyra* in conjugation, and of other algae that it was desirable to keep in stock for the use of students during the year, good results were obtained by boiling the material in a solution of acetate of copper before putting it into the preservative, whether formaldehyde or spirit. The compound of chlorophyll with the copper was formed very rapidly, in the hot solution, before any change due to decomposition had begun.

The extension of the method to larger specimens naturally followed, with very marked improvement in these as compared with the continued steeping in the cold solution. The hot solution penetrated more thoroughly and rapidly, and the green compound was formed in the tissues killed by the hot acid before decomposition had time to begin. Many plants were found to remain green when boiled in the solution of acetate of copper even of those that had always become brown when merely steeped in the cold solution. Another very great advantage of the use of heat is that it shortens very much the time required, the duration of treatment depending on the permeability of the tissues of each specimen. One or two minutes suffice for green seaweeds, and for submerged parts of vascular plants on which the cuticle is very thin, if present at all. The leaves and young stems of most plants require above five minutes' boiling. Those with thick cuticles and few stomata naturally require somewhat longer, up to 15 or 20 minutes in the more refractory specimens, the time to be allowed varying with the structure of the plants, and having to be estimated by experience.

After they have been boiled in the solution, and well washed in cold water to remove any surface deposits of acetate, they may be put at once into the preservative fluids, or they may be dried, if

suitable for that treatment. In either method they will usually show little change of colour when exposed to light. The green is often almost like that of the living plants; but in many it is more blue than natural, though retaining a decided green wherever chlorophyll was present in the living plant. In some cases where the specimens appeared brownish when removed from the boiling fluid the brown colour has been replaced by green in the spirit. Rarely does the colour become changed for the worse after permanent mounting.

When it is desired to preserve the green colour of dry specimens that are to be exposed to light they may be boiled in the solution of acetate, washed in pure water and dried, either by exposure to dry air, or in dry sand, or between sheets of botanical paper in the usual way, the method of drying being selected to avoid distortion in drying. Inflorescences of grasses give good results when simply exposed to dry air.

The colours of flowers and fruits are variously affected by this treatment. Some are destroyed by it, others, *e.g.*, the reds of rose-hips and various fruits, are largely retained. Occasionally curious results are obtained, as in the flowers of *Salvia splendens*, in which the colour disappeared from the corolla, but remained to a considerable extent in the calyx. The markings of variegated plants are well retained where these are pale on a green ground; and even where due to pigments or coloured sap these colours can often be recognised in the prepared specimens, even where much of their intensity has been lost.

An incidental advantage of the treatment is that the rapid killing of the tissues in the boiling solution lessens greatly the tendency to dismemberment by loss of leaves and even of branches, so troublesome in certain groups of plants, this result being more marked the shorter the time between the gathering of the specimens and the boiling. It is helpful in the treatment of such plants as *Sedum acre*, which are apt to remain alive for some time in the drying paper. It also helps to protect specimens against injury from fungi, so often hurtful in fluid preparations and in herbaria.

The experiments on the numerous plants selected by me for treatment were very carefully carried on by John Davidson, attendant in the Botanical Department of the University, and I take this occasion of expressing my appreciation of his services in this investigation.

Certain families of plants give less favourable results than one usually obtains, a dark exudation appearing on the leaves of some species. Probably further experiments will show means of overcoming some at least of these difficulties. Of course the treatment is not suitable for specimens so fragile that they would suffer injury from being boiled, even where the colour is well kept. But in many families the results are excellent, and in by far the most, even of the less favourable, the form is scarcely affected, the green parts remain clearly distinguished by their colour, and the educational value of the specimens is by so much the greater. It would be tedious to give a detailed statement of the effects of the treatment on the several plants subjected to it. The method is a

very simple one, and can be easily tried by anyone who wishes to test its worth. I believe it will be found useful. It is also economical in the saving of preservative fluids due to the lessened tendency to discolouration of these fluids by solution of substances (chlorophyll, &c.) from the specimens.

The method of treatment found to give the best specimens is as follows:—Saturate the volume (say one gallon) of commercial strong acetic acid with acetate of copper, shaking the bottle occasionally until no more will dissolve, some acetate being left at the bottom of the bottle. Pour off the clear solution, and add an equal volume of distilled or very soft water. Of this fluid enough is poured into an open enamelled or earthenware dish to allow the specimens for treatment to be submerged in it while it is being boiled over a suitable gas-burner. The specimens may be put at once into the boiling fluid, and should be kept sunk in it for periods as stated above, varying with their texture from two minutes to about twenty minutes. The fumes of boiling acetic acid are apt to be irritant to the eyes, nose, and throat, and also injure certain metals; hence it is well, if possible, to boil the specimens in a fume chamber, or in a place where the vapour can readily escape. The specimens should be lifted out with wooden forceps, and if they appear to have been boiled long enough they should be washed for a few minutes in water, and if necessary brushed or rubbed to remove deposits on their surfaces. They may then in most cases be prepared at once for permanent preservation, either in any of the usual preservative fluids, or dried.

IX.—DIAGNOSES AFRICANAE: XXI.

911. **Popowia Mannii**, *Baill. Adansonia*, vol. viii., p. 320 (1868) [Anonaceae]; descriptio emendata (auct. *T. A. Sprague*).

Frutex 1–1·5 m. altus (fide *Foster*). *Folia* obovato-oblonga usque ad elliptica, apice breviter obtuse acuminata, interdum leviter retusa, basi rotundata vel subcordata, 8–16 cm. longa, 3·5–6·5 cm. lata; petioli 2–5 mm. longi. *Petala* interiora in alabastro late ovata, demum subspathulata. *Ovarium* 1–3-ovulatum. Ceterum ut in descriptione Bailloniana.—*Clathrospermum Vogelii*, Oliv. in Fl. Trop. Afr., vol. i. (1868), p. 25, quoad specimen Mannianum. *C. Baillonii*, Scott Elliot in Journ. Linn. Soc., vol. xxx., p. 70 (1894). *Popowia Baillonii* Engl. et Diels in Engl. Monogr. Afr. Pfl., vol. vi., p. 48 (1901).

SIERRA LEONE. Bagru River, *Mann*, 809. S. NIGERIA. Oloke Meji, *Foster*, 312.

Ovaries from Mann's specimen had two ovules, those from Foster's only one, but there appears to be no other difference between the specimens apart from the degree of pubescence.

Perhaps too much stress has been laid on the number of ovules in *Anonaceae*. Evidently Bentham found only one ovule per carpel on first examining Mann's specimen, but found 2–3 ovules on subsequent examination. In a manuscript note by Bentham attached to the sheet bearing Mann's specimen is the statement "Ovula solitaria erecta"; the last two words have been subsequently crossed out by Bentham and "2–3, superposita" substituted.